VITAMIN B DETERMINATION AND REQUIREMENT WITH SPECIAL REFERENCE TO PROTEIN INTAKE

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DISSERTATION

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Faculty of Pure Science
of Columbia University.

BY

OLGA H. M. GLOY

NEW YORK
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ACKNOWLEDGMENT

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VITAMIN B DETERMINATION AND REQUIREMENT WITH SPECIAL REFERENCE TO PROTEIN INTAKE

In 1914, Braddon and Cooper[1] working with pigeons and considering the antineuritic vitamin and its relation to the different constituents in the diet stated that it was advisable to keep the proportion of antineuritic substance in the diet as high as possible and that as well as considering the diet's absolute content of antineuritic substance, its calorific value must be considered. Funk [2], also working with pigeons, stated that as the per cent of protein in the diet increases the amount of vitamin B needed decreases.

Drummond, Crowden and Hill [3] in 1922 have shown that in feeding growing rats on diets which contained high percentage of protein (up to 83%) in the form of casein, that the animals grew normally at first but afterwards not to the same extent as controls fed on a normal diet containing 20 per cent of protein. Their appearance seemed normal and on autopsy no marked differences could be detected from those on the normal diet. They failed to find any adequate reason for the difference. Later, Drummond and Reader[4] repeated this work feeding diets with more variations in the amounts of protein and again obtained similar results. At this same time, Hartwell [5] found that with lactating rats in order that the young should be in good condition as the protein in the mother's diet was increased, more vitamin B was needed. She proposed that there was some relationship between the protein and the amount of vitamin B required. As in some earlier work with lactating rats, Hartwell [6] had stated the hypothesis that the ill effects observed on high protein feeding was due to the excess of protein, Nelson[7] carried out some experiments to find whether these effects were due to excess of protein or deficiency of vita-

min B. From her work she concluded that on high protein diets the toxic symptoms observed in litters of rats were due to a deficiency of vitamin B. When the supply of vitamin B to such diets was increased no ill effects were observed and the mothers and litters were in good condition.

The purpose of the present work was to determine the vitamin B requirement with reference to protein intake.

The investigation was carried out with rats as the experimental animals and the growth-promoting water-soluble substance known as vitamin B, the substance considered. Variations in the percentage of protein in the basal diet used in testing for vitamin B were made to find out whether the increase in the protein had any effect on the length of survival of the "negative controls" and whether these variations affected the vitamin B requirement of the animal. ("Negative controls" is the term used in this laboratory for animals that are receiving the basal diet and no vitamin B.) Orange juice was used as the source of the vitamin and experiments were performed to estimate the vitamin B content of the juice.

In the first series of experiments a comparison of three basal diets with 12, 18 and 24 per cent of protein in the form of casein was made to find out if by varying the percentage of protein there are any appreciable effects on the length of survival of the animals. At th same time the vitamin B unit for orange juice was obtained. A tentative unit for vitamin B that has been worked out in this laboratory from the work of Sherman, Munsell and Burtis[8] is "that amount which fed daily to a standard test animal will result in net maintenance (neither loss nor gain) throughout the experimental period of 8 weeks." Further experiments were performed to see if altering the percentage of protein in the basal diet had any effect on the amount of vitamin B required for maintenance.

Experimental Procedure.

Young growing white rats, 28—29 days old, were given the experimental diets. Throughout all the experiments each animal was placed in an individual round galvanized iron wire cage, nine inches in diameter, with false bottom thus prevent-

ing access to the excreta. The cages were cleaned frequently. The experimental diet and fresh distilled water were fed *ad libitum* and a record of food consumption per week was kept. The rats were weighed at least once a week. The experimental period lasted for eight weeks. By means of a graduated pipette, accurately measured quantities of orange juice were fed daily excluding Sunday. This juice was prepared each day by filtering through a double thickness of cheese-cloth so that no pulp was included.

The composition of the three diets used is as follows:

	Diet 107A (per cent)	Diet 107 (per cent)	Diet 107B (per cent)
Casein	12	18	24
Butter fat	8	8	8
Cod liver oil	2	2	2
Osborne and Mendel Salt Mixture	4	4	4
Starch	74	68	62

The casein used was freed[9] from vitamin B by careful cold extraction with 60 per cent by weight alcohol. One liter of alcohol is added to 200 grams casein and the whole stirred with a mechanical stirrer for one half hour, then allowed to stand for five and a half hours, when it is then filtered on a Büchner funnel and washed with 500 cc. of 60 per cent (by weight) alcohol. Another liter of 60 per cent alcohol is added and again stirred for one half hour, after which it is allowed to stand for 18 hours, then filtered and washed with 500 cc. of 60 per cent alcohol and 500 cc. of 90 per cent (by weight) alcohol to facilitate air drying.

The butter fat used was freed from water, curd and salt by standing melted at a temperature below 50°, allowing to solidify, and then separating carefully. This was then remelted and filtered through paper within a hot water funnel. The cod liver oil was Squibb's. The salt mixture was that prepared according to the direction of Osborne and Mendel[10]; and the corn starch was a commercial product that had been shown previously to be free from vitamin B by Osborne, Wakeman and Ferry[11].

All the animals used in this work came from mothers that had been receiving Diet 13 (consisting of one-third whole milk powder, two-thirds whole wheat and sodium chloride, two per cent of the weight of the wheat, also known as Diet B in some of the publications from this laboratory), and only litters of six or more young rats were used.

A careful comparison of the three diets, containing 12, 18 and 24 per cent casein, could thus be made as in each litter one animal was chosen for each of the three diets and fed that only. Three other animals from the same litter were each fed one of the experimental diets and were given the measured amount of orange juice which was fed in small cups.

The initial weight of the animals used varied from 35—70 grams. Males and females were used indiscriminately, although, in grouping, as even a distribution of each sex as possible on each diet was made.

Length of Survival of Negative Controls.

The experimental basal diet 107, containing 18 per cent casein, is the one used in this laboratory when testing for vitamin B. It has been found to be adequate in all respects except for its vitamin B content. In altering the per cent of protein no difference was made in the caloric value of the diet, for carbohydrate which yields the same number of calories per gram as protein was the other component changed. The food consumption on the three diets was very similar and the animals receiving the diet containing 24 per cent of protein, ate it as readily as those in the other two groups with the lower amounts of protein.

For the first two weeks after being placed on the experimental diets the animals showed a slight gain in weight but at the end of the third week weighed less than the initial weight. The loss of weight continues until death and the average food consumption per week shows a decrease in the amount of diet eaten. The typical symptoms of vitamin B deficiency were observed in the animals on the three diets,—loss of vitality, the animals becoming very lethargic, very irritable, assuming a hunched position and in many cases showing distinct symp-

toms of polyneuritis. The rats also became extremely emaciated and felt very cold to the touch. All the animals were autopsied after death and the findings in all cases were similar. The average length of survival of the negative controls was for diet 107A (14 cases) 32.6 days, for diet 107 (12 cases) 31.1 days, and for diet 107B (14 cases) 32.1 days.

Curves are shown in Group D on Chart I and individual data in Tables I, II, and III for the animals on diets 107, 107A, and 107B, respectively.

TABLE I.

WEEKLY GAINS AND LENGTH OF SURVIVAL OF ANIMALS ON BASAL DIET 107 ONLY.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams	Days of Survival
19060	39	5	4	–3	–6	–7	–1			–8	37
19087	42	6	–3	–8	–5	–5				–15	33
19102	49	4	–4	–5	–8	–4				–17	32
19205	39	2	1	–3	–1	–6				–7	36
19213	45	–1	+1	–2	–6	–2				–10	31
19219	40	4	2	–4	–9	–1				–8	30
19249	38	1	–1	–6	–4					–10	29
19281	49	1	–1	–5	–9	–4				–18	32
19290	47	4	0	–4	–8	–4				–12	31
19439	43	9	–5	–8	–6					–10	25
19493	51	9	–5	–10	–8					–14	26
19511	56	0	–3	–6	–8	–1				–18	31
Average		3.7	–1.2	–5.3	–6.5	–3.8	–1			–12.3	31.1

TABLE II.

WEEKLY GAINS AND LENGTH OF SURVIVAL OF ANIMALS ON BASAL DIET 107A ONLY.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams	Days of Survival
19058	44	4	2	–4	–5	–3	–5			–11	44
19080	53	3	–5	–1	–8	–5	–4			–20	38
19086	39	3	–2	–5	–3	–7				–14	33
19200	48	1	–2	–5	–2	–5	–1			–14	36
19211	47	5	–2	–4	–7	–3				–11	31
19218	48	2	0	–5	–6	–4				–13	30
19244	47	2	–5	–2	–6	–5				–16	32
19253	37	2	–2	–3	–4					–7	27
19282	43	2	–4	–3	–7	–1				–13	31
19286	48	3	–2	–3	–12					–14	28
19434	52	8	–2	–9	–9					–12	27
19497	50	2	–5	–5	–9					–17	27
19513	51	–1	–5	0	–12					–18	29
19100	52	1	–2	–6	–3	–6	–5			–21	41
Average		2.6	–2.6	–3.5	–6.5	–4.3	–3.8			–14.3	32.6

TABLE III.

WEEKLY GAINS AND LENGTH OF SURVIVAL OF ANIMALS ON BASAL DIET 107B ONLY.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams	Days of Survival
19063	39	6	–2	–2	–4	–5	–6			–13	41
19081	51	5	–3	–5	–3	–6	–4	–2		–18	44
19084	44	3	–4	–4	–4	–7				–16	33
19105	46	–3	–1	–5	–5	–5				–19	32
19202	46	–2	–1	–3	–3	–7				–16	33
19212	46	–3	+2	–4	–6	–1				–12	30
19221	39	2	1	–2	–6	–3				–8	30
19247	41	3	1	–6	–4	–4				–10	32
19255	36	3	2	–3	–8					–6	28
19284	39	4	–1	–2	–8					–7	28
19292	44	3	0	1	–9	–4				–9	31
19442	49	11	–4	–7	–9					–9	28
19492	54	9	–8	–6	–7	–4				–16	30
19515	45	7	–6	–3	–6	–2				–10	29
Average		3.4	–1.7	–3.6	–5.9	–4.4	–5	–2		–12.1	32.1

From these results it can be seen that the length of survival of controls on the three Diets 107A, 107, and 107B, containing 12, 18, and 24 per cent of protein respectively, shows no significant difference. Thus in testing for vitamin B, a uniform result can be obtained on basal diets in which the protein varies from 12—24 per cent. where the increase of protein is balanced by a corresponding decrease in carbohydrate. No deleterious effect was shown when the highest per cent of protein was used.

Results of Feeding Graded Allowances of Vitamin B with Basal Rations of Different Protein Content.

Orange juice as sole source of vitamin B was fed in 1 cc., 2 cc., and 4 cc. portions to animals receiving the basal Diets 107, 107A, and 107B. Diet 107 is the usual basal diet used in this laboratory in testing for vitamin B content of foods and thus in feeding orange juice in different quantities to rats on this diet, the volume required to give maintenance could be found.

Seven animals were fed this diet and 1 cc. orange juice and the average length of survival was 39.4 days. The curve in Group C on Chart I shows the average gains of these seven animals. Table IV(a) shows the gain for each of these animals. The typical symptoms of vitamin B deficiency were observed in all the animals though the rats remained healthy looking longer than the controls that received no vitamin B.

At the same time seven other animals were fed the basal diet and 2 cc. orange juice. All of these animals lived throughout the 8 weeks experimental period. The animals showed small gains for the first three weeks after which there was a gradual decline. The rats showed signs of polyneuritis towards the last week of the experimetal period and were emaciated and irritable. The curve in Group B Chart I shows the average gain and Table IV(b) the gains of the individual animals.

Four animals were fed the basal diet and 4 cc. orange juice. The curve in Group A Chart I shows average gains and Table

IV(c) the gains for the individual animals. All the animals lived through the experimental period and were in fairly good condition at the end.

From these curves it can be seen that 4 cc. of orange juice supports maintenance whereas 1 cc. and 2 cc. are below maintenance. As these experiments were carried on at the same time, all the animals concerned received the same sample of orange juice and therefore the results are strictly comparable.

In the same way 1, 2 and 4 cc. of orange juice were fed to animals on the basal Diets 107A and 107B containing 12 and 24 per cent of casein respectively. As in the case with the animals fed basal Diet 107 and 1 cc. orange juice, the animals fed basal Diets 107A and 107B and 1 cc. orange juice did not live through the experimental period. The curves in Group C Chart I show the average gains of these animals.

Of seven rats on Diet 107A and 2 cc. orange juice, six lived throughout the eight week period. The curve in Group B Chart I shows the average gains and Table V(a) the gains for the individual animals.

Of six animals on Diet 107B and 2 cc. orange juice, all lived througout the 8 weeks. The curve in Group B Chart I shows the average gain and Table VI(a) the gains for the individual animals.

From these two curves it can be seen that 2 cc. orange juice was a sub-maintenance dose when the basal diets 107A and 107B were fed.

Four animals were fed basal Diet 107A and 4 cc. orange juice. All lived throughout the experimental period and were in fairly good condition at the end of the experiment. The curve in Group A Chart I shows the average gain in weight of these animals. Table V(b) shows the gains for the individual animals.

Five rats were fed Diet 107B and 4 cc. orange juice. They all lived throughout the experimental period and no differences were observed between these animals and those on either Diet 107 or 107A and 4 cc. orange juice. The curve in Group A Chart I shows the average gains and Table VI(b) the gains of the individual animals.

From these curves, in Group A Chart I, it can be seen that 4 cc. orange juice was sufficient to afford enough vitamin B for maintenance for the rats throughout the 8 weeks period. These results with 1 cc., 2 cc., and 4 cc. orange juice are similar to those obtained with Diet 107.

It can be concluded therefore that the amount of vitamin B that was sufficient for maintenance when the diet contained 18 per cent casein was not altered when the per cent of casein varied from 12—24 per cent.

TABLE IV(a)

WEEKLY GAINS AND LENGTH OF SURVIVAL OF ANIMALS ON DIET 107 + 1 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams	Days of Survival
19117	57	5	5	–5	–5	–2	–6	–6		–14	48
19220	40	4	5	–5	–4	–3	–2	–2		–7	45
19541	54	5	3	–8	–9	–2				–11	31
19556	47	8	–1	–5	–8	–6				–12	34
19583	46	10	–4	–5	–3	–5	–1			–8	37
19647	52	6	–4	–2	–7	0	–4	–1	–2	–14	52
19673	53	6	–6	–8	–7	–1				–16	29
Average		6.3	–.3	–5.4	–6.1	–2.7	–3.2	–3	–2	–11.7	39.4

TABLE IV(b)

WEEKLY GAINS OF ANIMALS ON DIET 107 + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19062	42	0	7	2	–1	–3	+1	–2	+1	+5
19103	48	5	3	–1	–3	–3	–2	–2	–3	–6
19118	52	1	6	3	–7	+1	–6	0	–5	–7
19206	38	–1	3	–3	0	–3	–2	–1	0	–7
19216	43	3	4	–1	–3	–6	–2	–1	0	–6
19226	40	3	9	–2	–2	–6	–3	+1	–6	–6
19291	46	1	3	3	–7	–2	–1	0	–1	–4
Average		1.7	5	+.1	–3.3	–3.1	–2.1	–.7	–2	–4.4

TABLE IV(c)

WEEKLY GAINS OF ANIMALS ON DIET 107 + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19088	41	1	6	3	0	0	1	–2	–1	7
19217	40	5	5	0	–1	–1	0	–2	0	6
19547	49	4	6	–5	–4	0	0	–1	0	0
19678	52	5	1	0	–4	–4	–3	–2	–1	–8
Average		3.8	4.5	–.5	–2.3	–1.3	–.5	–1.8	–.5	1.3

TABLE V(a)

WEEKLY GAINS OF ANIMALS ON DIET 107A + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19059	44	4	4	5	–5	–2	+1	–2	0	+5
19082	54	4	–4	0	–4	–4	–1	0	–2	–11
19101	49	1	3	–3	–3	–3	–2	–2	–3	–12
19141	72	5	–2	–5	–5	–2	–5	–5	–1	–20
19201	43	0	–1	–2	0	–1	–1	–1	0	–6
19225	41	1	2	2	–4	–3	–4	0	0	–6
19287	46	4	1	–1	–2	–3	–1	–6		–8
Average		2.7	.4	–.6	–3.3	–2.6	–1.9	–2.3	–1	–8.4

TABLE V(b)

WEEKLY GAINS OF ANIMALS ON DIET 107A + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19085	41	4	4	0	2	0	2	–5	–4	3
19133	62	1	–2	–1	+4	+1	–6	+5	0	2
19214	47	–2	+7	–2	0	–3	–1	–2	–1	–4
19254	37	4	4	5	–4	–2	0	–2	–2	+3
Average		1.8	3.3	.5	.5	–1.0	–1.3	–1	–1.8	1

TABLE VI(a)

WEEKLY GAINS OF ANIMALS
ON DIET 107B + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19064	37	4	2	2	–4	–2	+2	–2	0	2
19083	47	5	8	–3	–3	0	–3	–2	–2	0
19104	46	7	0	–3	–2	–4	+3	–3	0	–2
19145	65	6	1	–2	–4	–1	–4	–2	–2	–8
19223	35	7	7	–3	–2	–2	–3	–3	–1	0
19293	44	2	4	5	–6	–3	–2	–2	+1	–1
Average		5.1	3.7	–.7	–3.5	–2	–1	–2.3	–.7	–1.5

TABLE VI(b)

WEEKLY GAINS OF ANIMALS
ON DIET 107B + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19106	43	3	2	2	–1	–2	–2	–4	–2	–4
19129	72	8	–1	0	–1	+4	–9	+4	+2	7
19204	43	0	3	–2	+1	–1	+3	0	–2	2
19215	46	5	7	1	–6	–3	0	–2	+2	4
19258	36	5	4	4	–4	–1	–2	–2	–1	3
Average		4.2	3	1	–2.2	–.6	–2	–.8	–.2	2.4

The Effect of Higher Protein Diets on the Length of Survival of "Negative Controls."

Further experiments were carried out with diets containing 36 and 54 per cent of casein. Parallel with these, animals were given Diet 107 containing 18 per cent casein, as in the first series of experiments this diet was used as the standard on which the comparison of results was made. At the same time, in order to find if the vitamin B need was affected as the protein in the basal diet increased, orange juice was fed to young rats in accurately measured amounts as the source of the vitamin.

The selection of animals and experimental procedure was identical in every way with that described previously. Diet

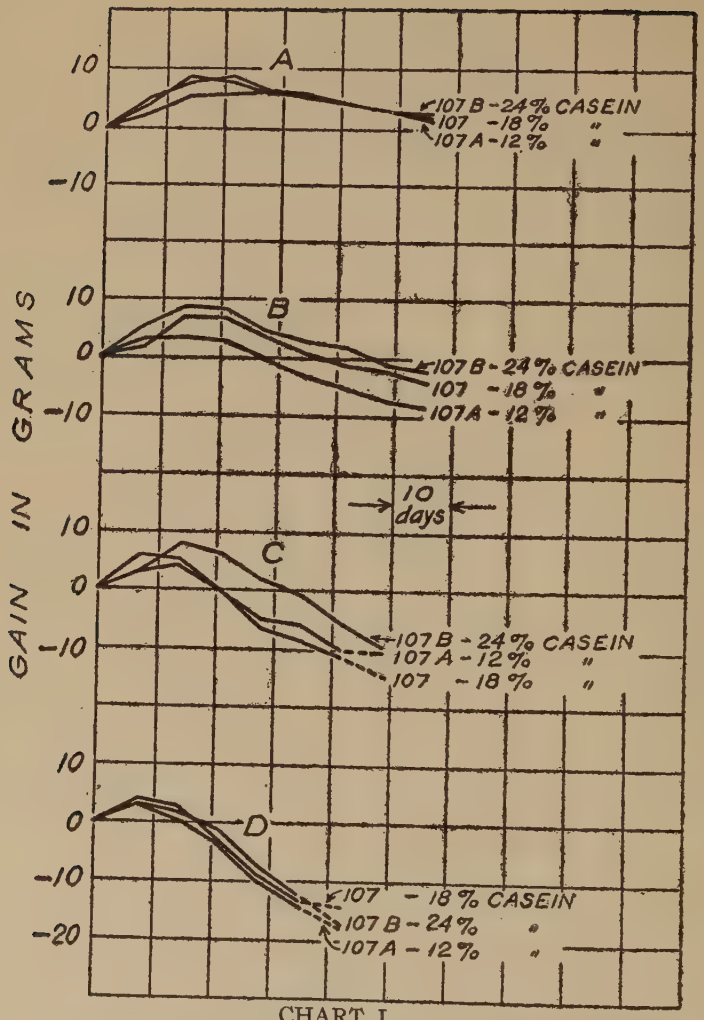

CHART I.

The average gain curves of animals fed on basal diets varying in protein content from 12—24% and the various portions of orange juice.

A gives the results for diet 107B (24% casein), 107 (18% casein), 107A (12% casein) and 4 cc. orange juice.

B for the same diets and 2 cc. orange juice.

C for the same diets and 1 cc. orange juice.

D for the basal diets only.

---- Indicates that less than half the animals were surviving.

107C contained 36 per cent and Diet 107D contained 54 per cent casein.

The composition of the Diets 107C and 107D was as follows:

	Diet 107C (per cent)	Diet 107D (per cent)
Casein	36	54
Butter fat	8	8
Cod liver oil	2	2
Osborne and Mendel Salt Mixture	4	4
Starch	50	32

Length of Survival of "Negative Controls" on Diets 107, 107C and 107D.

Fifteen animals of an average initial weight of 47.5 grams were placed on Diet 107 containing 18 per cent casein and the average length of life was 26 days. This result was therefore not quite consistent with that of the previous series of experiments where 31 days was the average length of survival. In this second series of experiments all the animals lost weight after the first week and showed the typical symptoms of vitamin B deficiency sooner than those in the first series. The average loss of weight in the two series was identical—13.3 grams.

MacArthur[12] in her work with animals on the basal diet, 107, found that the average length of life was from 29-30 days. This is in quite close agreement with the results reported here for rats in the first series of experiments. The reason for this appreciable difference in length of survival of the animals in the first and second series is hard to account for as no alteration had been made at any time in the method of preparation of food or the conditions under which the experiments were carried out. There is the possibility that there was a difference in the initial vitality of the animals. A careful study of the records of young growing rats on Diet 13, between the ages of 28-56 days, was made to find whether there was any seasonal variation in their growth. The figures used were

taken from records in this laboratory that ranged over a period of five years. The males and females were treated separately. These animals were at the same age as those used in the experimental work, but in the latter case the diet was deficient in vitamin B whereas Diet 13 has been well proved to be adequate for normal growth, reproduction and rearing of young.

Table VII shows the results of the analysis of the data for the males.

TABLE VII.

SEASONAL DIFFERENCES IN RATE OF GROWTH FROM 28 TO 56 DAYS OF RATS ON FULLY ADEQUATE DIET (MALES)

Months of Birth	No. of cases	Aver. Gain 28–56 days	Standard Deviation	Probable Error	Coefficient of Variation
January } February }	110	89.6	16.41	±1.04	18.3
March } April }	138	78.1	17.11	±0.99	21.9
May } June }	168	75.1	17.97	±0.93	23.9
July } August }	276	75.5	18.70	±0.71	24.8
September } October }	207	85.6	20.05	±0.93	23.4
November } December }	104	94.2	18.72	±1.22	19.9

Table VIII shows the results of the analysis of the data for the females.

TABLE VIII.

SEASONAL DIFFERENCES IN RATE OF GROWTH FROM 28 TO 56 DAYS OF RATS ON FULLY ADEQUATE DIET (FEMALES)

Months of Birth	No. of cases	Aver. Gain 28–56 days	Standard Deviation	Probable Error	Coefficient of Variation
January } February }	152	63.9	13.68	±0.74	21.41
March } April }	185	63.4	13.14	±0.64	20.7

Months of Birth	No. of cases	Aver. Gain 28–56 days	Standard Deviation	Probable Error	Coefficient of Variation
May } June }	191	59.4	12.45	±0.62	20.96
July } August }	356	60.5	14.05	±0.53	23.22
September } October }	299	66.9	13.87	±0.53	20.73
November } December }	126	67.4	14.14	±0.85	20.98

From tables VII and VIII it can be seen that there is a seasonal variation in the rate of growth of young rats on a fully adequate diet in the four weeks period after weaning. For animals born during the months May-June, the average gain in weight is less than that of animals born during the winter months, November-December. This shows that the young rats grow slightly more in the winter months than in the hot summer months. Both the males and the females showed the same seasonal variation, though the average increase in weight of the males is greater than that of the females.

At the same time that the rats were on the basal Diet 107, (18% casein) others were placed on the Diets 107C (36% casein) and 107D (54% casein). The length of survival of twenty-five animals on Diet 107C was 26.7 days, and of twenty animals on Diet 107D, 26.3 days. On the diet with 54 per cent casein difficulty was experienced in getting the animals to eat the basal diet as the high per cent of protein gave a diet not palatable to many. A number of the young rats died of starvation within the first week and all that lived lost weight the first week. They continued to lose weight until death and the average loss of weight was about 16.5 grams.

A small average gain was made by those on the diet with 36 per cent protein the first week, and then they lost weight until death. The animals on the two diets with the higher per cent of protein were in poorer condition than those on Diet 107 (18% casein). Those animals on the high protein diets excreted a urine that was very different in appearance from that

of the animals on Diet 107. It was a very thick brownish liquid with a disagreeable odor.

From the results of the average length of life of the "negative controls" on the three different Diets 107 (18% casein), 107C (36% casein), and 107D (54% casein), it could be concluded that the three basal diets were practically similar, on the other hand from the fact that there was difficulty in getting the animals to eat the high protein diets and the poorer condition of the animals on them, it would seem wise, in order to ensure the maximum of efficiency in testing for the vitamin content of foods, not to increase the protein in the basal diet as high as 54 per cent and better for it to be kept even lower than 36 per cent.

Vitamin B Requirement when the Protein in the Diet was Increased to 36 and 54 per cent.

In the first series of experiments, the amount of vitamin B (given as orange juice) required for maintenance was established for diets varying in protein content from 12—24 per cent. The same amounts, 1, 2, and 4 cc. of orange juice were again fed with the new Diets containing 36% protein, 107C, and 54% protein, 107D. In order to make the comparison more valuable, animals from the same litters were given the basal Diet 107, 18% casein, and the varying amounts of juice.

Some investigators, working with high protein diets and studying the vitamin need, have said that as the protein increased the vitamin B need also increased. To allow for this possibility, it was thought wise to feed some animals a higher amount of vitamin B, so 6 cc. of orange juice was fed as well as the other amounts given above.

The results obtained with Diet 107 (18% casein) and 1 cc., 2 cc., and 4 cc. orange juice in the second series in comparison with those of the first are shown below.

Animals Receiving Diet 107 + 1 cc. Orange Juice.

	Average Length of Survival—Days	Gain in Weight grams
1st Series	39.4	–11.7
2nd Series	38.3	–11.4

Animals Receiving Diet 107 + 2 cc. Orange Juice.

	Length of Survival	Gain in Weight grams
1st Series	8 weeks experimental period	–4.4
2nd Series	8 weeks experimental period	–4.6

Animals Receiving Diet 107 + 4 cc. Orange Juice.

	Length of Survival	Gain in Weight grams
1st Series	8 weeks experimental period	1.75
2nd Series	8 weeks experimental period	4.5

From this comparison it can be seen that with 1 cc. and 2 cc. orange juice the results obtained in the first and second series were comparable. With 4 cc., however, the animals in the second series made a slightly greater gain than those in the first series giving a result slightly above maintenance. Chart II shows the average gain curves of the animals on Diet 107 and 1, 2, and 4 cc. orange juice in the second series.

Four animals were fed Diet 107C (36% casein) and 1 cc. orange juice. The average length of survival was 39.3 days and typical vitamin B deficiency symptoms and polyneuritis were observed in all the rats.

Four rats were fed 107D (54% casein) and 1 cc. orange juice. These animals showed the same dislike for the high protein diet as the negative controls, though after the first week the food consumption increased but the rats lost weight until death. The average length of life was 40.75 days and the average loss in weight slightly greater than with those on 107C and 107.

Seven animals were placed on Diet 107C (36% casein) and 2 cc. orange juice. They all lived through the experimental period and the average gain was –4.9, almost identical with that of the rats on Diet 107 (18% casein) and 2 cc. orange juice.

Seven animals were fed Diet 107D (54% casein) and 2 cc. orange juice; five lived throughout the experimental period two dying at the end of seventh week. The average gain of weight in this group was –9.2 grams.

As with the basal diets where the per cent of protein varied from 12—24 per cent, in this case when the casein was increased to 36 and 54 per cent, the amount of vitamin B supplied by 1 cc. and 2 cc. of orange juice was a sub-maintenance amount. On the diet with 54 per cent of protein the animals that were fed the same amount of vitamin B as those on diets 36 and 18 per cent of protein showed an average loss in weight almost twice as great. This would seem to indicate that as the protein increased to 54 per cent that more vitamin B was required when the allowance of vitamin B fed was below maintenance level. Average gain curves are shown in Chart II.

Thirteen rats were fed Diet 107C (36% casein) and 4 cc. orange juice. These all lived throughout the 8 weeks experimental period, were in very good condition throughout and the average gain was 14 grams.

Twelve rats were fed Diet 107D and 4 cc. orange juice. The unpalatability of the unusually high protein diet was again demonstrated by the fact that for the first week most of the animals lost weight and the food consumption was low. However on becoming accustomed to the new food by the next week the food consumption had increased and all animals had gained in weight. They all lived through the experimental period and were in good condition throughout and on autopsy. The average gain was 7.8 grams.

With 54 per cent protein and 4 cc. orange juice the average gain was a little greater than with 18 per cent protein, thus the amount of vitamin B required as the protein was increased was practically the same as for 18 per cent casein in the diet.

With 36 per cent casein and 4 cc. orange juice the average gain was well above maintenance. From these results it would appear that as the protein in the basal diet was increased the amount of vitamin B necessary for maintenance did not show a corresponding increase.

With the animals on 6 cc. orange juice and Diets 107, 107C, and 107D, it is interesting to note that the average gain for the 8 weeks period was 23.8, 25.3, and 24.3 grams respectively. These animals were all receiving the same amount of vitamin

B and yet, although the protein content of the diet varied from 18—54 per cent, the average gains were practically the same.

This would still further strengthen the conclusion that as the protein in the diet was increased up to 54 per cent, the amount of vitamin B required was not greater than that needed when the diet contained 18 per cent protein.

Average gain curves for animals on the diets with protein content varying from 18—54% are given in Chart II. Individual data are shown in the Tables IX to XIV.

TABLE IX(a)

WEEKLY GAINS OF ANIMALS
ON DIET 107 + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19834	43	2	4	–4	–5	–3	–1	0	+1	–6
19851	41	7	3	–2	–4	–3	–1	0	+1	+1
19997	42	8	–1	–2	–2	–4	–2	+1	0	–2
20066	52	2	7	–1	–3	–3	–2	0	–2	–2
20432	42	8	3	–4	0	–8	–1	–5	–2	–9
20475	47	2	–4	–3	–3	–2	–1	+1	0	–10
Average		4.8	2	–2.7	–2.8	–3.8	–1.3	–.5	–.3	–4.7

TABLE IX(b)

WEEKLY GAINS OF ANIMALS
ON DIET 107 + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
20024	38	4	2	0	–1	–1	0	0	+1	5
20128	54	3	5	–2	–1	–2	–2	4	–5	0
20259	39	6	4	0	1	2	–2	0	–4	7
20327	39	0	3	–2	0	3	2	0	0	6
20365	57	6	12	–5	–2	–1	–3	–2	–1	4
20463	43	3	–1	5	3	1	0	–4	–2	5
Average		3.7	4.2	–.7	0	+.3	–.8	–.3	–1.8	4.5

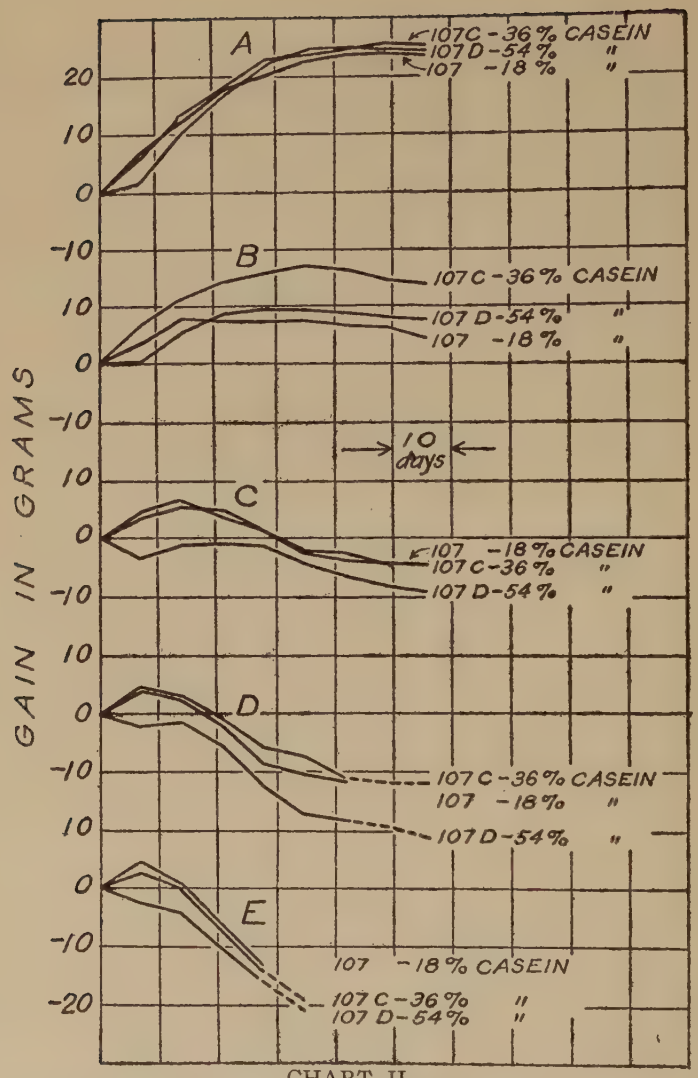

CHART II.

The average gain curves of animals fed on basal diets varying in protein content from 18—54% and the various portions of orange juice.

A gives the results for diet 107 (18% casein), 107C (36% casein), 107D (54% casein) and 6 cc. orange juice.

B for the same diets and 4 cc. orange juice.

C for the same diets and 2 cc. orange juice.

D for the same diets and 1 cc. orange juice.

E for the basal diets only.

---- Indicates that less than half the animals were surviving.

TABLE X(a)

WEEKLY GAINS OF ANIMALS
ON DIET 107C + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19832	45	0	3	–3	–5	–1	0	–1	+1	–6
19856	36	0	–4	+5	–2	–3	0	–2	+2	–4
19926	47	14	+8	–1	–5	–4	–1	–5	0	+6
19995	50	6	0	–6	–2	–4	–3	1	0	–8
20062	55	5	1	1	0	–6	–1	–3	1	–2
20430	47	0	1	–3	1	–6	+3	–3	–1	–8
20470	44	1	4	–1	–4	–6	+2	–4	–4	–12
Average		3.7	1.9	–1.1	–2.4	–4.3	0	–2.4	–.1	–4.9

TABLE X(b)

WEEKLY GAINS OF ANIMALS
ON DIET 107C + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19860	50	3	6	3	2	–1	6	1	4	24
19864	56	7	2	5	–1	2	1	0	2	18
19987	43	12	0	4	2	4	–4	–3	–3	12
20022	40	9	2	1	0	4	1	0	0	17
20027	47	6	–1	2	–2	–1	–1	0	0	3
20053	45	10	5	2	–4	+1	0	–1	–3	10
20123	61	3	7	2	1	1	0	–1	0	13
20257	45	4	8	3	7	2	1	–8	–3	14
20325	49	13	5	4	1	2	–4	–5	–1	15
20341	43	4	7	3	7	3	–2	0	0	22
20371	54	8	4	0	3	0	0	–2	–4	9
20403	50	3	9	3	4	0	–4	–3	–2	10
20461	44	5	6	4	2	0	–1	–1	0	15
Average		6.7	4.6	2.7	1.7	1.3	–.5	–1.8	–.8	14

TABLE XI(a)

WEEKLY GAINS OF ANIMALS
ON DIET 107D + 2 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19830	46	–14	3	3	–2	–1	–2	0	–2	–15
19852	44	–4	–1	1	–5	–1	–2	–5		–17
19928	43	–3	0	2	3	–1	2	2	–3	+2
19993	57	+3	5	–2	–1	–7	–3	–4	0	–9
20064	53	+6	–2	–1	0	–4	0	–1	0	–2
20427	46	–9	5	1	2	–4	–2	–3	–1	–11
20428	43	–2	3	–1	0	–5	–1	–6		–12
Average		–3.3	1.9	.4	–.3	–3.3	–1.1	–2.4	–1.2	–9.2

TABLE XI(b)

WEEKLY GAINS OF ANIMALS
ON DIET 107D + 4 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
19859	51	–4	2	4	1	0	1	2	3	9
19866	51	7	6	8	0	0	–2	–3	5	21
19929	35	–1	4	2	3	0	3	0	1	12
20019	41	–2	0	1	0	–3	1	1	1	–1
20030	37	–4	1	2	0	2	1	0	1	3
20051	46	8	6	2	–1	–1	2	1	–4	13
20126	57	0	5	–3	–2	–8	–4	0	–3	–15
20263	38	–4	6	4	3	1	–2	–2	–3	3
20328	44	6	9	5	2	0	–1	–4	–1	16
20338	46	6	2	4	3	2	1	–2	–2	14
20368	51	–1	11	4	4	4	–5	2	–1	18
20467	43	–6	5	4	2	0	–1	0	–3	1
Average		.4	4.8	3.1	1.3	–.3	–.5	–.4	–.5	7.8

TABLE XII

WEEKLY GAINS OF ANIMALS ON DIET 107 + 6 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
20260	33	–1	6	4	3	2	4	4	2	24
20330	28	9	7	7	3	2	3	1	–3	29
20366	56	9	4	3	2	0	–4	–2	–3	9
20405	44	9	7	8	2	4	0	–3	+3	30
20464	40	7	4	3	4	4	3	3	–1	27
Average		6.6	5.6	5.0	2.8	2.4	1.2	.6	–.4	23.8

TABLE XIII

WEEKLY GAINS OF ANIMALS ON DIET 107C + 6 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
20236	52	7	8	0	6	0	0	–1	0	20
20239	42	3	5	6	2	4	–3	+3	–1	19
20258	41	3	6	5	5	–1	+9	2	–1	28
20326	41	10	5	5	6	2	1	0	–3	26
20340	32	5	3	4	6	8	0	2	2	30
20372	49	3	12	6	0	0	0	2	1	24
20404	50	12	11	4	4	–1	–1	–4	1	26
20462	43	5	8	7	5	0	2	2	0	29
Average		6	7.3	4.6	4.3	1.5	1	.8	–.1	25.3

TABLE XIV

WEEKLY GAINS OF ANIMALS ON DIET 107D + 6 cc. ORANGE JUICE.

Rat No.	Initial Weight grams	Weekly gains during 8 weeks experimental period—grams 1	2	3	4	5	6	7	8	Total Gain grams
20232	53	4	11	6	1	7	–2	–3	–2	22
20233	52	3	8	7	3	5	–1	+2	–2	25
20264	38	0	11	7	8	1	4	–1	+1	31
20329	40	3	4	8	5	2	0	2	+1	25
20339	44	3	5	3	8	7	0	3	0	29
20369	48	–3	13	8	5	1	–5	+2	0	21
20409	38	0	7	5	5	–3	1	–2	0	13
20468	40	4	7	9	4	1	4	–1	0	28
Average		1.8	8.3	6.6	4.9	2.6	.1	.3	–.3	24.3

Conclusions.

I. In a diet devoid of vitamin B but adequate in all other respects, it was found that on varying the per cent of protein from 12—24 that no appreciable difference in the length of survival of negative controls was obtained. Thus a basal diet with the protein ranging from 12 to 24 per cent was found to be suitable when testing for vitamin B content of foods. This justifies the conclusion that, when the per cent of protein in the basal diet containing 18 per cent is increased by feeding a high protein food or lowered by feeding a high energy food as a source of vitamin B within the range 12—24 per cent, the results obtained are identical.

II. Four cc. of orange juice gave maintenance in the standard test animal through 8 weeks experimental period when the basal diet contained 18 per cent of casein. The vitamin B in 2 cc. and 1 cc. of orange juice was a sub-maintenance amount. The average loss of weight of the animals on 2 cc. was –4.4 grams and the average length of life of those animals on 1 cc. was 39.4 days as against 31.1 days for those receiving no vitamin B (negative controls).

III. When orange juice was fed to those animals on diets containing 12 and 24 per cent casein, the results obtained agreed with those described for the basal diet of 18 per cent protein. Therefore, in increasing the amount of protein in the basal diet, no increase in the amount of vitamin B required for maintenance was necessary nor was the effect observed to follow the feeding of a definite sub-maintenance allowance essentially altered in any way.

IV. Although the length of survival of negative controls on diets with 36 and 54 per cent protein was similar to those on 18 per cent, the general condition of the animals was not so good. In testing for the vitamin B of a food it would seem wise not to increase the protein in the basal diet as high as 54 per cent nor even as high as 36 per cent.

V. When orange juice was fed as a source of vitamin B, the amount required to give maintenance when the diet contained 12—24 per cent protein was found to give a greater gain in weight throughout the 8 weeks experimental period when the diet contained 36 and 54 per cent protein. When the amount of vitamin B was given in a higher dosage the average gains on diets with 18, 36, and 54 per cent casein were almost identical. From these results it may be concluded that as the protein in the diet was increased, the vitamin B requirement was not correspondingly increased.

Bibliography.

[1] Braddon, W. L. and Cooper, E. A. British Medical Journal, **I,** 1348 (1914).

[2] Funk, C. Compt. Rend. Soc. Biol. **92,** 997.

[3] Drummond, J. C., Crowden, G. P. and Hill, E. L. G. J. Physiology, **56,** 413 (1922).

[4] Drummond, J. C. and Reader, V. B. J. Physiology, **59,** 472 (1925).

[5] Hartwell, G. A. Biochem. J. **19,** 1074 (1925).

[6] Hartwell, G. A. Biochem. J. **15,** 140, 563 (1921).

[7] Nelson, P. M. J. Home Economics, **18,** 383 (1926).

[8] Sherman, H. C., Munsell, H. E. and Burtis, M. P. Unpublished Data.

[9] Sherman, H. C. and Spohn, A. J. Am. Chem. Soc. **45,** 2720 (1923).

[10] Osborne, T. B. and Mendel, L. B. J. Biol. Chem. **37,** 572 (1919).

[11] Osborne, T. B., Wakeman, A. J. and Ferry, E. L. J. Biol. Chem., **39,** 35 (1919).

[12] MacArthur, E. H. Dissertation, Columbia, 1926.

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